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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: June 28, 1957

1. Name of Investigator: Edward W. Pelikan, M.D.

2. Title: Associate Professor of Pharmacology

3. Institution & Address: Department of Pharmacology and Experimental Therapeutics
Boston University School of Medicine
80 East Concord Street, Boston 18, Massachusetts

4. Project or Subject: A study of structure-activity relationships among drugs which affect nicotine-sensitive physiological mechanisms.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Introduction.

1. Although the gross pharmacological actions of nicotine have been described in detail, there is a remarkable lack of knowledge of how and why nicotine affects the cells it does. We wish to study the mechanisms by which nicotine acts and to attempt to learn what aspects of the chemical structure of nicotine are responsible for each of its major actions. We believe this study can best be pursued by a comparative investigation of the actions of drugs with a wide range of chemical structures, all of which drugs possess actions in common with nicotine. In this study, the known actions of nicotine will be used as a standard for comparison; the biological systems on which nicotine acts will function as indicator systems; the major experimental variable will be variation in chemical structure of a series of drugs.

2. The understanding of the effects of drugs on autonomic and somatic nervous system activity has been confused by the relative non-selectivity of action of agents, the great selectivity of action of which has been assumed. We intend to use the lack of selectivity of these agents as the factor crucial to our experiments.

a. It is well known that nicotine is a compound with little selectivity and specificity of action. It is known that in the reflex arc, nicotine may stimulate sensory receptors, modify the integrative action of the central nervous system, influence ganglionic transmission, and alter the ac-

tivity of effector systems (at least in the case of skeletal muscle). Nicotine also has direct effects on the adrenal medulla and on isolated chromaffin masses, and on the posterior pituitary gland to cause the release into the circulation of substances which can affect a wide variety of tissues. The effects of nicotine are further complicated and made difficult to interpret since:

1. Nicotine given in large doses may diminish or abolish at least some of those activities stimulated by smaller doses.

2. Tachyphylaxis is developed toward at least some of the actions of nicotine.

3. Alteration of the state of the organism by any drug, including nicotine, induces compensatory reflex activity.

4. Even in a single tissue nicotine, present in a given concentration, may modify several functions of the tissue in opposite ways. Taugner, for example, has shown that the same dose of nicotine which will facilitate the homolateral flexion reflex of the decerebrate cat will inhibit the patellar reflex.

b. The relative lack of selectivity of action of some of the "autonomic drugs" is less well known and less well studied than is true for nicotine. It is well known that acetylcholine has both "nicotinic" and "muscarinic" effects, and that even muscarine has some "nicotinic" effects. Drugs used to produce ganglionic blockade are known to have variable amounts of activity at the neuromuscular junction, and in the central nervous system (Chen); at both of these sites ganglionic blocking drugs act antagonistically to nicotine.

Sympathomimetic amines have actions at at least four sites which are also sensitive to nicotine: Sympathomimetic amines can stimulate receptors in the area of the carotid body (Eyck and Comroe), can have effects on the central nervous system, can modify ganglionic transmission (Matthews, Trendelenburg, and others), and can alter the activity of effector organs, including skeletal muscle (Huidobro, and others).

We have already reported the results of our comparison of the effects of nicotine and a series of sympathomimetic amines on the isolated rabbit jejunum and the isolated frog rectus abdominis muscle. It was found that nicotine and derivatives of phenethylamine and tyramine had similar actions on the jejunal strips; in general, derivatives of phenethylamine and tyramine manifested their sympathomimetic actions only after the strips had been treated with a ganglionic blocking agent in a concentration sufficient to abolish the actions of nicotine. It could not be determined with certainty that epinephrine, norepinephrine and 3-hydroxytyramine had nicotine-like actions in this tissue. In the isolated frog rectus abdominis preparation, phenethylamine and tyramine derivatives had actions qualitatively like those of nicotine: all the compounds produced contracture of the muscle which could be prevented by tubocurarine, and tachyphylaxis developed to the actions of all of the agents. Epinephrine, norepinephrine and 3-hydroxytyramine failed to produce contracture of the frog muscle, but, rather, blocked the actions of nicotine on the muscle.

In our studies using the superior cervical ganglion of the cat, we have observed that all of a series of sympathomimetic amines had effects qualitatively similar to those of nicotine in inhibiting ganglionic transmission. The sympathomimetic amines most potent in this respect were those which also possessed the greatest sympathomimetic effect: epinephrine was three times as potent as nicotine in producing ganglionic blockade. Under these conditions

of our experiments none of the agents, including nicotine, facilitated ganglionic transmission. Matthews, who used a different experimental procedure, found that at least the sympathomimetic amine, isopropylnorepinephrine, could produce such facilitation. Kewitz and Reinert used still another experimental method and found that epinephrine blocked ganglionic transmission and facilitated the ganglionic response to injected acetylcholine, but that amphetamine blocked the effects of acetylcholine and facilitated the effects of preganglionic nerve stimulation.

B. Specific proposal.

1. We propose to test the hypothesis that compounds with qualitatively similar actions on nicotine-sensitive mechanisms have chemical-structural features in common. We hope to identify such common chemical properties among nicotine, sympathomimetic amines, and ganglionic and neuromuscular blocking agents. The qualitative similarities in action and site of action (lack of selectivity) of the compounds we hope to study implies that similarities exist among the mechanisms by which the drug molecules interact with cells. Specifically, the inference seems justified, and susceptible to experimental testing, that similar parts of grossly different molecules react similarly with identical cell receptors.

2. Once chemical-structural elements common to drugs which act on nicotine-sensitive mechanisms have been identified, we hope to study quantitative differences in these common elements. We hope to explain quantitative differences in drug action and selectivity in terms of quantitative differences in the common elements. We wish to investigate the mechanism of action of nicotine by means of a comparative study of the relationship between structure and action among a number of compounds which have actions similar to one or more of the actions of nicotine.

C. Methods.

1. In vivo methods.

a. We anticipate using two in vivo methods: one method to permit the study of drug effects on transmission of nerve impulses in sympathetic ganglia, one method to permit studying the effects of drugs on nerve impulse transmission at the skeletal neuromuscular junction. Four questions are to be answered on the basis of the results of these experiments:

1. Have all of the drugs to be studied qualitatively similar, but quantitatively dissimilar, actions, or are the actions of some of the drugs qualitatively dissimilar from those of other agents?

2. Are there agents which only facilitate transmission, others which only inhibit transmission, and others which, like nicotine, may both increase and decrease function depending on the dose of the agent used?

3. To what extent are apparent differences in drug action to be referred to differences in experimental procedure (parameters of stimulation, criterion of drug effect, dose range studied, etc.) and to what extent may they be referred to actual differences in action among the drugs themselves.

4. What similarities of chemical structure can be found among agents with qualitatively similar actions on nicotine-sensitive mechanisms?

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b. Methods to be used to study the effects of drugs on transmission of impulses through sympathetic ganglia.

1. Cats will be pithed while under ether anesthesia; artificial respiration will be provided to the cat and the effects of the ether will be allowed to disappear. Arrangements will be made to permit recording the contractions of one of the nictitating membranes, and to record the arterial blood pressure of the cat. Provision will be made for intravenous administration of drugs.

2. Perfusion of the superior cervical ganglion, on the side of the nictitating membrane the contractions of which are to be recorded, will be accomplished using one of the modifications of the method of Kibjakow. Circulation to the nictitating membrane will be preserved by anastomosis of the cut proximal end of the ipsilateral external carotid artery to the cut distal end of the contralateral lingual or external carotid artery. Perfusion of the ganglion will permit the administration of drugs to the ganglion alone, or to the nictitating membrane alone, and permit the evaluation of the effects of drugs which are active at both sites.

3. The preganglionic fibers to the perfused ganglion will be prepared so that the fibers may be stimulated electrically at predetermined parameters of stimulation. The postganglionic fibers from the ganglion will be prepared so that ganglionic and postganglionic potentials may be displayed on the oscilloscope according to the method of Eccles. Arrangements will be made to stimulate postganglionic fibers electrically.

4. We shall observe, record and quantitate the changes in postganglionic action potentials and the responses of the nictitating membrane (to various rates of preganglionic stimulation) which follow the administration to the ganglion of a wide range of preselected, graded doses of the compounds to be studied. We hope thaf quantitate our data by measuring the effect of the drugs to be studied on the frequency-effect curve of the postganglionic potentials and on the contraction of the nictitating membrane as described by Rosenblueth and Cannon.

5. All precautions will be observed in order to verify the site of action of the drugs, and to ensure that the actions observed are, in fact, attributable to the drugs. Both negative and positive control drug injections will be used; positive control drugs will be drugs which are known to affect ganglionic transmission by mechanisms other than those postulated for nicotine, e.g., anesthetic agents (Exton), and morphine (Trendelenburg).

c. Methods to be used to study the effects of drugs on transmission of impulses through the skeletal neuromuscular junction.

1. The responses of the leg muscles of the fowl to indirect stimulation are useful in differentiating among drugs which influence neuromuscular transmission (a) By causing transient depolarization of the neuromuscular junction, (b) By preventing depolarization of the junction by acetylcholine released from motor nerve terminals, (c) By potentiating the effects of acetylcholine by inhibiting cholinesterase at the junctional region. The criteria for these drug effects are, respectively, (a) Alteration of the resting tension of the muscle, (b) Diminution of the amplitude of contraction of the indirectly stimulated muscle, and (c) Alteration in the myogram of single muscle twitches evoked by nerve stimulation.

2. We propose to use this method of study, which has been described previously (Pelikan, Smith and Unna; and Theraleff and Unna): Myograms of the tibialis anterior and soleus muscles of the unanesthetized chicken are to be recorded mechanically. Contraction of the muscles is induced by electrical stimulation of the appropriate branches of the sciatic nerve. Drugs may be administered either intravenously or by retrograde injection into the ischiadic artery. Various parameters of stimulation may be used to permit the determination of frequency-effect curves.

3. The methods of analysing the data will be analogous to those used in studying the effects of the drugs on ganglionic transmission. The qualitative nature of the drug effect will be determined by inspection of the myogram. The effects of the drugs will be quantitated by determining their effects on the frequency-response curves of the muscles.

2. In vitro methods.

a. We hope to classify the drugs studied according to chemical structure and qualitative effects on nicotine-sensitive mechanisms in vivo. Such a classification should permit at least tentative identification of those structural features responsible for the qualitative action of the drugs. We hope to study in vitro quantitative differences among the drugs and relate these differences to quantitative differences in their chemical properties.

b. Ariens has emphasized that quantitative differences in apparent biological activity of compounds which act after combination with cell receptors (as nicotine is presumed to do) must be interpreted in terms of two fundamental properties of the drug which may vary independently of each other and which are dependent on the chemical structure of the drug: (1) Intrinsic biological activity, and (2) The affinity of the pharmacophoric groups of the drug for the cell receptors. We propose to study the intrinsic activity and affinity of nicotine and the compounds to be studied for the nicotine-sensitive mechanisms of the isolated frog rectus abdominis muscle.

c. Our preliminary results indicate that the nicotine-sensitive mechanism of the frog rectus abdominis muscle provides a good model-system on which to study the properties of drugs which act on ganglia and other neuromuscular junctions. The isolated frog muscle is eminently suitable for precise, quantitative studies under a wide variety of experimental conditions.

d. The molecular species (ionized, unionized) responsible for the stimulatant and blocking effects of the drugs on the frog muscle will be determined by observing the effects of alterations of the pH of the medium on the apparent potency of the drugs. Similar methods have been used to determine the molecular species responsible for the actions of barbiturates, alcohols, and local anesthetics. The intrinsic activity of the optimally effective molecular species will be determined for the agents which we study.

e. We hope to measure the affinity of the drugs for the cell receptors by determining "wash-out" times of the drugs from the tissue as a measure of affinity. Preliminary studies have shown us that the "wash-out" curves for sympathomimetic amines and nicotine follow the equation for the pseudomonomolecular reaction; it might be predicted that such a curve would describe "wash-out" rates when the rate was determined by the rate of dissociation of the drug-cell complex. Our results so far indicate that the slopes of "wash-out" curves, i.e., the apparent dissociation constants, differ from compound to compound in the case of sympathomimetic amines. "Wash-out" experiments will be performed at several temperatures in order to verify, by determining the Q10, that the wash-out process is limited in rate by a chemi-

6. Budget Plan:

Salaries	REDACTED
Expendable Supplies	2,000
Permanent Equipment	2,000
Overhead (15% of total)	1,365
Other Misc., including travel	600
Total	REDACTED

* E.W. Pelikan REDACTED
Research
Assistant

7. Anticipated Duration of Work:

Two years; the budget plan above applies only to the first year of research.

8. Facilities and Staff Available:

Facilities and staff of the Department of Pharmacology and Experimental Therapeutics of the Boston University School of Medicine. The staff members are competent and well equipped to aid the course of this study, especially insofar as special knowledge of anatomic and histological, biochemical and physical chemical methods might be required, and insofar as special techniques might be required to record EK and study cardiovascular phenomena. No salaries are requested for persons who might aid the work in this way.

9. Additional Requirements:

None

10. Additional Information (including relation of work to other projects and other sources of supply):

It is anticipated that the research will follow the outline described above. As the study progresses, minor modifications of experimental design and technique may be necessary; the general plan of the study and its goals will not, of course, be changed.

This research program is a direct outgrowth and extension of previous investigations conducted by the applicant. These investigations have included studies of the pharmacology of the neuromuscular junction in several species, studies of the structure-activity relationships among neuromuscular blocking agents, and studies of the actions of nicotine and sympathomimetic amines on isolated intestinal strips, the frog rectus abdominis muscle, and on the superior cervical ganglion of the cat.

Application for support of this work has not been made to any other agencies.

The applicant will spend about three-fourths of his time on these investigations.

Signature /s/ Edward W. Pelikan, M.D.
Director of Project

/s/ Reimus Rosen
Business Officer of the Institution

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CURRICULUM VITAE OF EDWARD W. PELIKAN

DATE AND PLACE OF BIRTH: REDACTED

DEGREES:
B.S. (University of Illinois, 1948)
M.S. (University of Illinois, Department of Pharmacology, 1950)
M.D. (University of Illinois College of Medicine, 1951)

TRAINING AND EMPLOYMENT:
Research Assistant, Department of Pharmacology, University of Illinois College of Medicine, 1948-1951.
Instructor, Department of Pharmacology, University of Illinois College of Medicine, 1951-1953.
Interne, The Presbyterian Hospital of the City of Chicago, 1951-1953.
Special Projects Officer, Office of Naval Research, U.S. Navy, 1953-1955.
Assistant Professor, Department of Pharmacology, University of Illinois College of Medicine, 1953-1955.
Assistant Professor, Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania
Associate Professor, Department of Pharmacology, Boston University School of Medicine

SOCIETY MEMBERSHIPS: REDACTED

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